Contents

1.0 Purpose/Scope

This procedure provides a standardized method for the operation of the **Aerotech 6™ Viable Microbial Particle Sampler** and similar microbial culture plate sampling devices.

The sampler provides a method for surveys to collect aerobic species of bacteria and fungi for Indoor Air Quality investigations and personnel exposure monitoring for etiologic agents. This procedure is designed to meet NIOSH Method 0800 specifications for sampling indoor and outdoor air for viable microorganisms.

The Single-Stage Viable Particle Sampler is an aluminum device held together by three spring clamps and sealed with two O-ring gaskets. The unit consists of an inlet cone, a jet classification stage, media agar plate, and a base plate. The jet classification stage contains 400 precision drilled holes. When air is drawn through the sampler, multiple jets of air passing through the drilled holes direct any airborne particles toward the agar surface where collection occurs based on the principle of inertial impaction.

2.0 Responsibilities

2.1 Use of the sampler is limited to persons who act under the direction of a competent

hazard assessment person and have demonstrated the competency to satisfactorily use the sampler, as evidenced by experience and training set in Section 7.

2.2 Personnel that perform data collection and hazard assessments with this sampler are responsible to follow all steps in this procedure.

2.3 The data collected using this sampler must have an appropriate evaluation of the hazard and risk by a cognizant Industrial Hygiene professional from the organization conducting the sampling.

3.0 Prerequisites

3.1 **Area Access, when needed**:

3.2.1 Contact the Facility Support Representative or Technician to obtain approval to enter radiological areas. Verify if a Work Permit or Radiological Work Permit is needed or is in effect. If so, review and sign the permit.

3.2.2 Use appropriate PPE for area or wear hearing protection when levels are unknown.

4.0 Precautions

4.1 Hazard Determination:

4.1.1 The operation of this sampler does not cause exposure to any radiological hazards.

4.1.2 The sampler design does not cause significant ergonomic concerns in routine use.

4.1.3 The sampler does not generate Hazardous Waste. The sampling plates are sent to the analysis laboratory for processing and ultimate disposal. Any unused or exposed sampling plates that are not sent to an off-site laboratory for analysis are to be decontaminated as per section 6. A small amount of Isopropanol is used to wipe the sampler between samples. The bulk will evaporate into the ambient air. A paper towel can be used to speed the drying and can be disposed of in the trash.

4.1.4 Observe the work planning and operational controls set in Job Risk Assessment SHSD-JRA-05.

4.1.5 By its very nature, the sampler may be used in areas where mold growth may be present. Workers should be aware of the potential for exposure to microbiological contamination (e.g. fungi & bacteria) and take necessary precautions for personal protection and minimize disturbance of building materials to prevent airborne release of spores or other antigens.

4.2 Personal Protective Equipment:

4.2.1 In areas, where the release of very high levels of spores or antigens are expected (e.g. visible growth), respiratory protection should be worn. The minimum level of protection should be a NIOSH approved N95 filtering face-piece respirator.

4.2.2 Other appropriate PPE may be needed for the area being entered. Check with your ES&H Coordinator or Facility Support representative.

6.0 Procedure

6.1 Equipment:

- Air sampling pump capable of 30 liters per minute (Andersen 10-709)
- Aerotech 6™ Sampler
- Sterile Agar plates
- Tripod
- Stopwatch
- Isopropanol
- Paper towels

6.2 **Media:** Order the agar media plates from the analytical laboratory or a media supply company (15 x 100 mm plate) Media that is typically used are as follows:

- Malt Extract Agar (MEA) –Fungi
- Tryptic Soy Agar (TSA) – Bacteria
- Blood Agar Bovine – Human Bacterial Pathogens

- Keep Agar plates refrigerated until ready to use.
- Allow the plates to warm up to room temperature before taking a sample (approx. 20 minutes).
- Do not remove the lid from the plate at anytime except during sampling.

6.3 **Calibration:**

6.3.1 Run the sampling pump for at least 30 seconds prior to setting the flow rate. Calibrate the pump prior to taking samples and again at the end of sampling. The pump and sampler do not need to be calibrated between each sample.

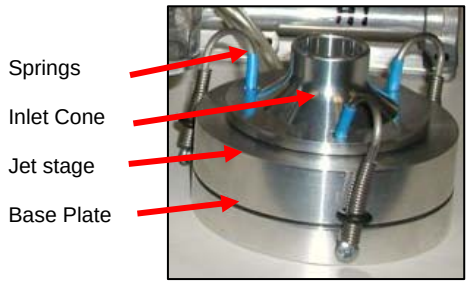
6.3.2 Calibrate the pump with the Aerotech 6™ sampler inline at 1 cu /ft/ min (28.3 liters/min). Calibrate using IH SOP of IH 75150: *DryCal* or IH75160**:** Singer DTM-200 Dry Test Meter.

6.4 **Selecting sampling areas** (for Indoor Air Quality investigations). Choose sampling locations that will help identify the source of molds, including (the appropriate) of:

- at least two locations outdoors,
- an area that building occupants frequently enter that does NOT have an IAQ problem,
- the desk or work location of each person with a IAQ complaint or symptoms,
- near the supply of the HVAC system or wall air conditioner,
- an isolated area where dust/mold might accumulate,
- areas with water staining or mold growth,
- an area with a soft, porous surface (such as carpet), and
- a "blank" that is taken to the sampling site

6.5 **Assemble the Aerotech 6 ™ Sampler with media:** It is recommended to wear disposable gloves during sampling to avoid contamination of media. Otherwise, clean your hands with a hand sanitizer or soap and water before sampling and when moving between sampling areas.

- Release the three retaining springs from their indents on the "inlet cone".
- Lift the inlet cone from the "jet classification stage".
- Lift the "jet classification stage" from the "Base Plate".
- Remove the agar plate from the media storage bag/box and carefully remove the cover plate.



- Place the agar on the three raised metal pins of the "Base Plate". (Avoid touching the surface of the media with hands.)
- Reassemble the sampler with the agar plate inside.
- Attach one end of tubing to the intake of the vacuum pump and the other end to the inlet of the sampler.

6.6 **Sampling:**

- Set the timer to an appropriate time depending on environmental conditions (sampling time is between 2-5 minutes, **3 minutes** at 1 cu ft/min **is usually ideal)**.
- Turn on the pump and start the timer simultaneously.
- After the sample is taken, unhook the three spring clips and remove the jet classification stage and inlet cone.
- Remove the Agar plate. Secure the lid onto the dish with masking tape or laboratory film (avoid using electrical, packing, transparent, and duct tape).
- Write the sample number on the bottom of the agar plate.
- Place the agar plate into a sterile sample bag to minimize contamination.

6.7 **Post sampling Clean-up:** Wipe all exposed surfaces of the Aerotech 6™ with 70% Isopropyl alcohol and allow to air dry between sampling and at the end of sampling**.** Wear Nitrile gloves during wiping of surfaces.

6.8 **Recording readings:** Use the BNL Microbial sampling Form (Attachment 9.1) to record readings (see the IH SOP web page for the most recent version).

6.9 Prepare a Chain of Custody form to accompany the plates as per IH51300.

6.10 Ship the plates to the off-site analysis laboratory with adequate packing material to protect the plates. Insert icepacks in the shipping container, however, the sample plates should not come into direct contact with the ice to avoid freezing the media and invalidating the tests.

6.11 **Analysis of results:** A competent person should write a hazard evaluation report that evaluates the survey data and summarizes the potential for occupational exposure and compliance with ACGIH *Biologically Derived Airborne Contaminants.* Guidance provided in OSHA Technical Manual Section III: Chapter 2 states: **"**The identification of predominant taxa, or at least fungi, is recommended in addition to determining the number of colony-forming units/m^3 of air (cfu/m^3). During growing seasons, outdoor fungus-spore levels can range from 1,000 to 100,000 cfu/m^3 of air. Contamination indicator: **1,000 viable colony-forming units in a cubic meter of air.** Levels in excess of the above do not necessarily imply that the conditions are unsafe or hazardous. The type and concentrations of the airborne microorganisms will determine the hazard to employees." See excerpts of an internet report from the International Center for Toxicology and Medicine in Attachment 9.3.

6.12 **Reporting and Recordkeeping:** See IH 6050): Reporting Exposure Monitoring Results for more requirements on the content and reporting deadlines. Ensure that a copy of the hazard evaluation report is sent to the IH Laboratory and is included in the ESHQ Directorate Recordkeeping system under IH89 (for etiologic agent sampling) or IH 97 (for Indoor Air Quality Investigations) as per IH60200 Records Management & Document Retention.

6.13 **Disposal of excess agar plates:** Make a 10% household bleach solution with tap water in a gallon size jar/beaker. Label the contents on the container. Place the agar plates into the solution for 24 hours. At the end of 24 hours, with PVC or Nitrile gloves, lift the plates from the solution, leaving the media in the solution. Pour the used 10% bleach solution into a toilet and flush.

7.0 Implementation and Training

7.1 Training prior to using this meter includes a demonstration of proper operation of the instrument based on training, education, and experience. All persons must have met the qualification criteria for JTA IH97 IAQ Assessor or IH89 Biohazard Assessor set in IH50300: BNL IH Program and IH Group Training & Qualification Matrix.

7.2 Personnel are to document their training using Attachment 9.2, the Job Performance Measure Completion Certificate. Qualification on this JPM is required on a 3 year basis, providing the professional is monitoring sources frequently.

8.0 References

8.1 Aerotech 6™ Operating Manual.
8.2 OSHA TECHNICAL MANUAL SECTION III: CHAPTER 2
8.3 ACGIH Threshold Limit Values 2006
8.4 BNL SBMS Subject Area *Indoor Air Quality*.
8.5 BNL SBMS Subject Area *Biohazards in Research.*
8.6 Ronald E. Gots, M.D., Ph.D. International Center For Toxicology And Medicine (ICTM);**Correcting Mold Misinformation, Internet Article, 2006.**

BROOKHAVEN NATIONAL LABORATORY Safety & Health Services Division **INDUSTRIAL HYGIENE GROUP** Standard Operating Procedure: Field Procedure	NUMBER **IH89300** REVISION **Final Rev1**
Subject: **Aerotech 6™** **Viable Microbial Particle Sampler**	DATE **06/27/06** PAGE **7** of 13

9.0 Attachments

9.1 **Sample of a Microbial Sampling form**
9.2 **Job Performance Measure (JPM) Completion Certificate**
9.3 **Published Ambient Microbe Levels**

10.0 Documentation

Document Development and Revision Control Tracking		
PREPARED BY: *(Signature and date on file)* **N. Sanchez** **Author** **Date 05/04/2006**	**REVIEWED BY:** *(Signature and date on file)* **R. Selvey** **SHSD IH Group Leader** **Date 05/11/06**	**APPROVED BY:** *(Signature and date on file)* **R. Selvey** **SHSD IH Group Leader** **Date 05/11/06**
ESH Coordinator/ Date: *none*	Work Coordinator/ Date: *none*	SHSD Manager / Date *none*
QA Representative / Date: *none*	Training Coordinator / Date: *none*	Filing Code: **IH52**
Facility Support Rep. / Date: *none*	Environ. Compliance Rep. / Date: *none*	Effective Date: **05/11/06**
ISM Review - Hazard Categorization ☐ High ☐ Moderate ☒ Low/Skill of the craft	Validation: ☐ Formal Walkthrough ☐ Desk Top Review ☐ SME Review Name / Date:	Implementation: Training Completed: Tracked in BTMS Procedure posted on Web: 05/11/06 Hard Copy files updated: 05/11/06

Revision Log		
Purpose: ☐ Temporary Change ☐ Change in Scope ☐ Periodic review ☐ Clarify/enhance procedural controls Changed resulting from: ☐ Environmental impacts ☐ Federal, State and/or Local requirements ☐ Corrective/preventive actions to non-conformances ☒ none of the above Section/page and Description of change: Added Attachment 9.3 for more information to be used in the evaluation of results.		
R. Selvey 06/27/06(signature/date on file) SME Reviewer/Date:	SME Reviewer/Date:	SME Reviewer/Date:

BROOKHAVEN NATIONAL LABORATORY

SHSD Industrial Hygiene Group

MICROBIAL AIR MONITORING

IH89300

File Code: IH63

DATE:	DEPT:	BLDG/AREA:	SURVEYOR:
SAMPLER:	MEDIA:	FLOW: A PRE: POST: L/min	FLOW: B PRE: POST: L/min
PRE CALIBRATION DATE:	POST CALIBRATION DATE:	CALIBRATION METHOD:	
PLATE STORAGE:	ANALYSIS DATE:	ANALYSIS METHOD:	
DESCRIPTION OF SAMPLING AREA:			___Central HVAC ____Wall A/C ___None ___Radiator (___Steam or ___Electric)
FLOOR: ___CARPET ___NON-CARPET	CEILING: ___PLASTER ___SUSPENDED	WATER DAMAGE/MOLD GROWTH:	
OUTDOOR TEMP: °F °C	INDOOR TEMP: °F °C	OUTDOOR RH: _% DewPt:	INDOOR RH: % DewPt:

SAMPLING DATA						ANALYSIS DATA									
SAMPLE NUMBER	PUMP I.D.	TIME OF DAY (hh:mm)	RUN TIME (mm:ss)	SAMPLE VOLUME (L)									TOTAL CFU	CFU/M^3	CFU/FT^3

Sample:

See IH SOP Web page for current version

Use this form or any other similar form to record samples. The off-site analysis report can replace the "Analysis Data" portion of this form.

BROOKHAVEN NATIONAL LABORATORY

SHSD Industrial Hygiene Group

MICROBIAL AIR MONITORING

IH89300

File Code: IH63

SAMPLING DATA						ANALYSIS DATA									
SAMPLE NUMBER	PUMP I.D.	TIME OF DAY (hh:mm)	RUN TIME (mm:ss)	SAMPLE VOLUME (L)	LOCATION								TOTAL CFU	CFU/M^3	CFU/FT^3

MICROBIAL AIR MONITORING

IH89300

File Code: IH63

BROOKHAVEN NATIONAL LABORATORY

HP-IHP-89300
IH89300
Attachment 9.2

Environmental, Safety, Health & Quality Directorate
SHSD Industrial Hygiene

Aerotech 6™ Viable Microbial Particle Sampler
Job Performance Measure (JPM) Completion Certificate

Candidate's Name	Life Number:

Knowledge of the Principles of IAQ Investigations

Criteria	Qualifying Standard	Unsatis-factory	Recov-ered	Satisf-.actory
Hazard Analysis	Understands the need to perform a hazard analysis of the sampling area and potential exposure to the sampler.			
Personal Protective Equipment	Understands the need to be aware of potential IAQ causing agent hazards to sampler and knows how to determine the need for PPE.			
Sampling Protocol	Understands the exposure monitoring logic necessary to appropriately select sampling locations to accurately measure worker, public and environmental exposure potential.			
	Demonstrates knowledge in the reasons to select sample locations to maximize the effectiveness of the sampling and determine if problems exist.			
	Demonstrates knowledge of the correct media to order and use in sampling.			
Analysis of data	Understands the need to perform analysis on the sampling data to assess potential exposure to the worker and public to recommend corrective actions as necessary.			

Practical Skill Evaluation: Demonstration of Sampler Operation

Criteria	Qualifying Performance Standard	Unsatis-factory	Recov-ered	Satisf-.actory
Sampling Equipment	Knows where equipment needed for the procedure is located and how to properly sign it out.			
Survey Technique	Demonstrates the proper way to use the sampler and handle the media.			
Record forms	Shows how to correctly and completely fill out all forms associated with this SOP.			
Data Analysis	Shows how to correctly have the data analyzed and compared to occupational exposure limits or other appropriate reference points.			
Employee Notification	Knows how to timely and properly notify workers and management of over exposure.			

Employee: I accept the responsibility for performing this task as demonstrated within this JPM and the corresponding SOP.

Candidate Signature:	Date:

Evaluator: I certify the candidate has satisfactorily performed each of the above listed steps and is capable of performing the task unsupervised.

Evaluator Signature:	Date:

Selected Published References on Ambient Microbe Levels

OSHA Technical Manual Section III: Chapter 2: The identification of predominant taxa, or at least fungi, is recommended in addition to determining the number of colony-forming units/m^3 of air (cfu/m^3). During growing seasons, outdoor fungus-spore levels can range from 1,000 to 100,000 cfu/m^3 of air. Contamination indicator: **1,000 viable colony-forming units in a cubic meter of air.** Levels in excess of the above do not necessarily imply that the conditions are unsafe or hazardous. The type and concentrations of the airborne microorganisms will determine the hazard to employees.

Excerpts from Ronald E. Gots, M.D., Ph.D. Principal, International Center for Toxicology and Medicine (ICTM) regots@ictm.com www.ictm.com: *Correcting Mold Misinformation:*

- One should be concerned about concentrations of mold detected in indoor ambient air that are greater than 100 to 200 CFU/m3 or greater than 1000 spores/m3. There are no established threshold levels at which adverse health effects are documented. Therefore, a comparison of mold concentrations commonly found in indoor ambient air and those measured in the outdoors is an appropriate beginning guideline. Unless extensive water-damage is evident, the majority of residential and commercial structures have indoor ambient air levels below those detected in the outdoors. However, this varies with time of year, location and mold genera. Gots, R.E., Layton, N., and Pirages, S.W. "Indoor health: Background levels of fungi." AIHAJ, in press.; Gots, R.E., Gots, B.A., Spencer, J. "Proving causes of illness in environmental toxicology: 'sick buildings' as an example." Fresenius Envir Bull 1 (1992): 135-42.

- Average concentrations in indoor ambient varies seasonally and geographically Indoor ambient air in 820 residences without any health complaints averaged 1,252 CFU/m3 and the associated average outdoor level is reported as 1,524 CFU/m3 (Gots et al., in press). For 85 homes with concentrations reported as total spore counts, the average ranged from 68 to 2,307 spores/m3 for the indoor air and a range of 400 to 80,000 spores/m3 in outdoor ambient air. Shelton, B.G., Kirkland, K.H., Flanders, W.D., and Morris, G.K. 2002. "Profiles of airborne fungi in building and outdoor environments in the United States." Appl Environ Microbiol 68:1743-1753.

- Mold spore levels in cities around the country show remarkable geographic and seasonal variation that must be considered when making such comparisons. Examples of outdoor seasonal variability observed in 2001 include (NAB 2001): National Allergy Board. 2001. Pollen and mold counts. www.aaaai.org.:

City	March to June	September to December
St. Louis, MO	395 to 24,500 spores/m3	5,266 to 68,855 spores/m3
Las Vegas, NV	8 to 673 spores/m3	15 to 186 spores/m3
Albany, NY	9 to 1,534 spores/m3	1,075 to 18,005 spores/m3
Santa Barbara, CA	544 to 33,090 spores/m3	767 to 555,833 spores/m3

- Occupational exposures, via handling materials of natural origin, can be extremely high. At sawmills, maximum airborne concentrations have been reported as 1,500,000 CFU/m³). Duchaine, C., Meriaux, A., Thorne, P.S., and Cormier, Y. 2000. "Assessment of Particulates and Bioaerosols in Eastern Canadian

Sawmills." Am. Ind. Hyg. Assoc. J. 61:727-732.

- A study of differences in air concentrations on farms with and without disease revealed an average exposure concentration of 120,000,000 spores/m³ on the control farms (Daily spore levels associated with adverse health effects were at least ten times greater than that (1,200,000,000 spores/m3). Malmberg, P., Rask-Andersen, A., and Rosenhall, L. 1993. "Exposure to Microorganisms Associated with Allergic Alveolitis and Febrile Reactions to Mold Dust in Farmers." Chest 103:1202-1209.

- Air concentrations in spawning sheds on mushroom farms have been reported as high as 100,000 spores/m³; even greater concentrations are detected at other areas on these farms (Fungi detected in the breathing zone of workers in a municipal waste composting facility reach levels of 8,200,000 CFU/m3). Lacey, J., and Crook, B. 1988. "Fungal and Actinomycete Spores as Pollutants of the Workplace and Occupational Allergens." Ann Occup Hyg 32:515-533.